UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF PENNSYLVANIA

POLYSCIENCES, INC., Plaintiff, v. JOSEPH T. MASRUD, Defendant.	Civil Action No. **DECLARATION OF LEENA MOL THURUTHIPPALLIL, PH.D.**

I, Leena Mol Thuruthippallil, Ph.D., of full age, hereby declare, under penalty of perjury, as follows:

1. I have an undergraduate degree in Biology from Mahatma Gandhi University, India, a Ph.D. in Toxicology from Ehime University, Japan and a MBA from Temple University, PA, USA. I am Director of Lab Products at Polysciences, Inc. ("Polysciences"). I have held this position since July 8, 2019, and have been responsible for all the Life Science and Material Science products under the Lab Products Division. Some of my key responsibilities are as follows: a) Responsible for the profit and loss of the Lab Products business; b) Identify and grow key segments that drove growth; c) Manage key accounts, and other sales initiatives; d) Serve as a trusted advisor for large/strategic customers, and build and maintain strong customer (internal & external) relationships; e) Responsible for developing and implementing product specific catalog/bulk, distribution and channel sales strategies, work closely with external suppliers and

distributors; f) Deliver innovative and profitable products and services; g) Define the product/MGPP roadmap, making judicious choices on priorities; h) Lead consistent execution of product lifecycle processes from concept to launch, driving value-based prioritization of product development initiatives to ensure maximum ROI; i) Drive the marketing initiatives, set up the marketing goals and Go-to-market strategies; j) Coordinate implementation of product and process improvements to deliver amazing customer experiences; k) Responsible to define, track, and communicate metrics that help understand how well initiatives are meeting objectives; l) Execute channel strategies to support current and future products; m) Oversee inventory control and revenue recognition; and n) Lead, manage, and mentor a team of highly talented product managers, sales, operational and customer service managers (accountable for strategic hires, training and development).

2. I submit this Declaration based upon my personal knowledge, information, and belief in support of Polysciences' Motion for a Preliminary Injunction.

3. Transfection is the process of introducing nucleic acids into eukaryotic cells (cells that contain a nucleus and organelles, which are enclosed by a plasma membrane). The main purpose of transfection technology is to study the function of genes or gene products, by enhancing or inhibiting specific gene expression in cells, and to produce recombinant proteins in mammalian cells. Transfection overcomes the challenge of introducing negatively charged molecules (e.g., DNA and RNA) into cells with a negatively charged membrane.

4. Common transfection reagents include chemical, lipid, and electroporation. Chemical methods neutralize the negative charge of DNA, facilitating its uptake. Lipid-based reagents can also coat DNA while forming micelles or bubbles entrapping the DNA. Electroporation makes the membrane more permeable transiently, allowing DNA to enter the

cell. Chemicals like polyethylenimine ("PEI"), a cationic (positively charged) polymer whose linear form has been described as the most efficient to transfect a wide range of cell lines and thus is broadly used in transient gene expression (Delafosse et al. *Data in Brief* 8 (2016) 456-460). This process makes it easier for the DNA:transfection reagent complex to cross the membrane.

5. Occasional changes in the genetic makeup of cultured cells allow them to proliferate indefinitely, making them effectively immortal. Such cell lines used in transfection experiments include HEK-293, CHO, COS, HeLa, and a variety of other eukaryotic cell lines.

6. Polysciences is one of three commercial suppliers who has the demonstrated ability to provide quality PEI transfection reagents for the research, development, and commercial supply of biological treatments for humans. (The other two companies that offer PEI transfection reagents are Polyplus-transfection and Sigma Aldrich.) In the past 60 years, Polysciences has been recognized as a trusted and well-respected manufacturer of high-purity monomer and polymers products for the scientific community. There are hundreds of research articles mentioning the use of Polysciences PEI transfection reagents. Polysciences has a collection of small- to mid-scale manufacturing processing and packaging equipment, allowing us to manufacture a broad array of products, including PEI transfection reagents, that are widely used for various scientific applications.

7. PEI can exist in different forms (solid and liquid of varying concentrations) and configurations, including branched or linear structures with different molecular weights. Likewise, PEI products can be produced in a variety of ways, resulting in varying quality and efficiencies with respect to different applications or uses. Thus, production of each type of PEI products requires careful design and development in order to have consistent quality and performance. Polysciences manufactures various PEI products made from PEI polymers with

different molecular weights, with branched and linear structures, provided in powder or liquid form, and cGMP and non cGMP versions. Polysciences has spent over 15 years and millions of dollars in total to develop these various PEI products.

8. Polysciences provides ready-to-use, powerful, trusted, and cost-effective transient transfection reagents, including Transporter™ 5 and PEI MAX. Transporter™ 5 Transfection Reagent is based on a proprietary PEI derivative. The structure of Transporter™ 5 condenses DNA into positively charged complexes which enter the cell by endocytosis. Transporter™ 5 works exceptionally well for transfection of HEK-293, CHO, COS, HeLa, insect cell lines (Sf9 and Sf21) and a variety of other eukaryotic cell lines. Transporter 5™ produces high titers without any supplementation. Transfection efficiencies of Polysciences's transfection reagents are tested by in-house quantitative performance assay instead of other commercial qualitative assays that cannot ensure the high titers or greater concentration that most scientists require.

9. PEI MAX is also known as PEI 40K or PEI 22K in free base. Compared to common PEI 25K that contains 4-11% residual propionyl groups, PEI MAX has a fully depropionylated structure that allows the polymer backbone to more strongly interact with DNA, which means each batch performs consistently higher.

10. The appropriate selection of a transfection reagent is extremely important as it has a significant impact on the overall process/final yield. There are several studies that observed high transfection efficiencies and low cytotoxicity of Polysciences linear PEIs (e.g., PEI MAX) (Aravindan *et al*., *Int J Pharm*, 378(1-2), 201-210 (2009); Dai *et al*., *Biomaterials*, 32(6), 1694-1705 (2011); Nimesh *et al*., *Int. J. Pharm*. 337, 265–274 (2007)). PEI MAX is nearly fully deacylated, which makes it a very effective but less cytotoxic transfection reagent.

11. For transfection efficiency, the study by Gu, *et al.* compared transfection efficiencies of PEI MAX to those of PEIpro and Lipofectamine-LTX (Life Technologies) and found that an approximately 3-fold titer increase using PEI MAX compared to PEIpro (Gu, *et al.*, *Cell Gene Therapy Insights* 2018; 4(S1), 753-769). In another study by Grieger, *et al.*, it was found that "PEI Max was superior in transfection efficiency than PEI on our adherent and suspension HEK293 cells" (Grieger, *et al.*, *Mol Ther*. 2016 Feb; 24(2): 287–297).

12. With regard to cytotoxicity, Delafosse *et al*., 2016 investigated the cytotoxicity of three PEI-based transfection reagents (PEI MAX, PEI 25K, and PEIpro) in 293-6E and CHO-3E7 cell lines using the PI fluorescent dye exclusion assay and observed PEI MAX as the less toxic reagent for both cell lines compared to PEIpro.

13. Polysciences PEI reagents also have high scalability and flexibility. They are suitable for a wide range of cell lines/types. It is suitable for stable as well as transient transfection and ensures high viral/protein titers in both adherent and suspension cultures. As many viral vector manufacturing processes use suspension cell lines derived from Human Embryonic Kidney (HEK 293) and Chinese Hamster Ovary (CHO), cells we have shown comparative data for these cell lines.

14. There are several critical factors that influence biotherapeutic manufacturers to use Polysciences PEI in their process development through clinical trials and the commercial manufacturing process. Polysciences transfection reagents are of high-quality (R&D and GMP grades); less cytotoxic but with high transfection efficiency; highly reproducible viral/protein titers at any scale (smaller culture dish to large scale bioreactors); flexible (suitable for stable and transient transfection with a wide range of cell lines); all materials are sourced and manufactured in US in an ISO certified facility under an ISO 13485 Quality System; and are cost effective.

15. My comparison of the protocols, product specifications and performance indicates high similarity between Polysciences's and Serochem's transient transfection reagents, such as Transporter™ 5 (Polysciences) and PEI Prime (Serochem). In my opinion, such similarity cannot be an accident. In particular, the protocol of Suspension Cell Culture Transient Transfection with PEI Prime (Serochem) uses the same mixing order of the reagents for transient transfection (DNA – PEI – cells), the same DNA concentration (100 µg DNA per 100 ml or 1 µg/ml), the same PEI starting concentration (1 mg/ml), and similar cell density as specified in the "Protocol For Suspension Cells" using Polysciences's Transporter™ 5. Also, Serochem recommends not to freeze PEI Prime solution formulated from the powder and to store it at 4°C for 6 months. This is exactly what we recommend to our customers as in Mathew Griffin's (previous LP product manager who reported to Joseph Masrud) opinion PEI MAX forms precipitates upon a freeze-thaw process, which could adversely affect the transfection efficiency of the product. Its highly impossible for Serochem to know as in my opinion they unlikely have had enough time to do a product stability study.

16. As of July 13, 2020, Serochem compared its PEI product transfection performance to Polysciences PEI "Max" transfection performance. Serochem's website stated as follows:

> "PEI Prime™ is a great transfection reagent for CHO transient gene expression (TGE). We evaluated PEI Prime™ against a popular transfection-grade PEI using an SEAP Reporter Assay comparable to that described in https://doi.org/10.1016/j.jbiotec.2016.04.028.

Delafosse et al., *J. Biotechnol.* (2016) 227:103-111:" This scientific article compares the transfection efficiency of Polysciences LPEI and PEI "Max" and Polyplus Transfection PEIpro products. It is my opinion that Serochem relied on this article to compare its PEI product

performance to Polysciences PEI superior product performance. Serochem had removed this research article from its website.

I declare under penalty of perjury under the federal laws of the United States that the foregoing is true and correct.

Dated: July 23, 2020 By: 

Leena Mol Thuruthippallil, Ph.D.